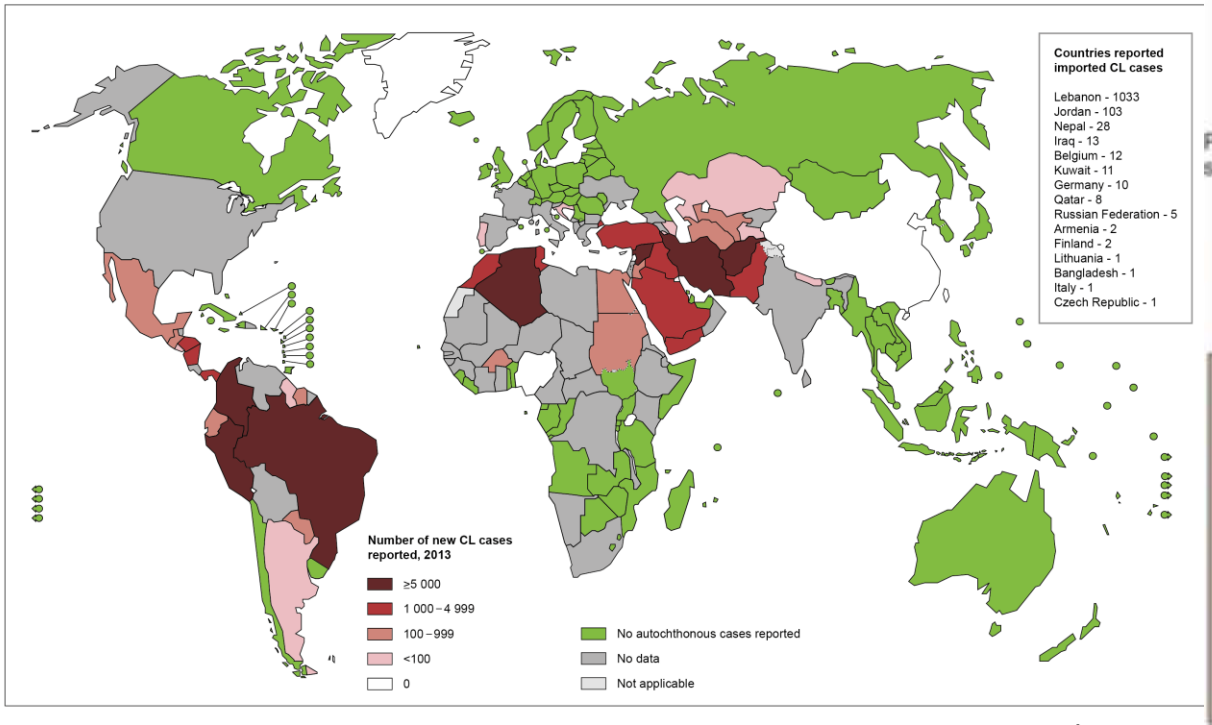
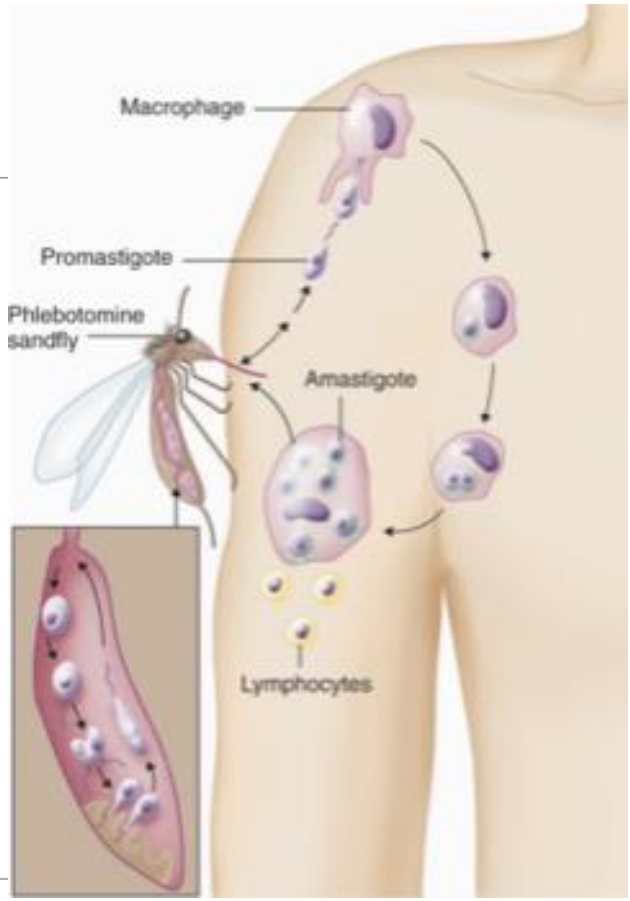
Drug Delivery by Tattooing with a PMU machine to treat Cutaneous Leishmaniasis

Cdr Dr Stef Stienstra, scientific advisory board MT.Derm & Amiea, Germany
& CBRN Subject Matter Expert Dutch Armed Forces, 1-CMI Command
Dr Anny Fortin, Dafra Pharma R&D, Belgium & Dept. of Biochemistry, McGill University, Canada

Leishmaniasis is a vector-borne disease caused by obligate intra-macrophage protozoa of the Leishmania species.

Status of endemicity of cutaneous leishmaniasis, worldwide, 2013



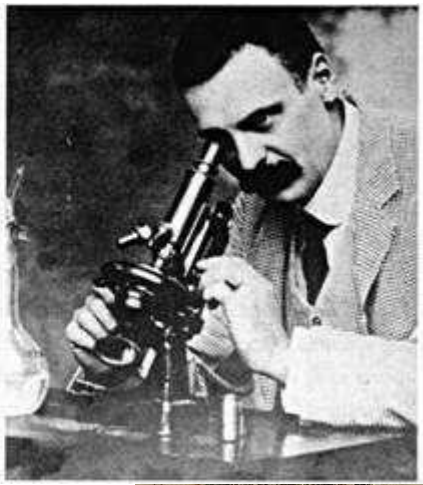


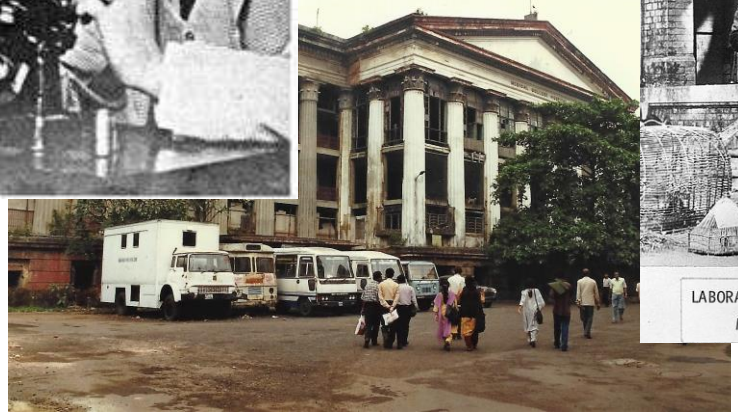
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. © WHO 2015. All rights reserved

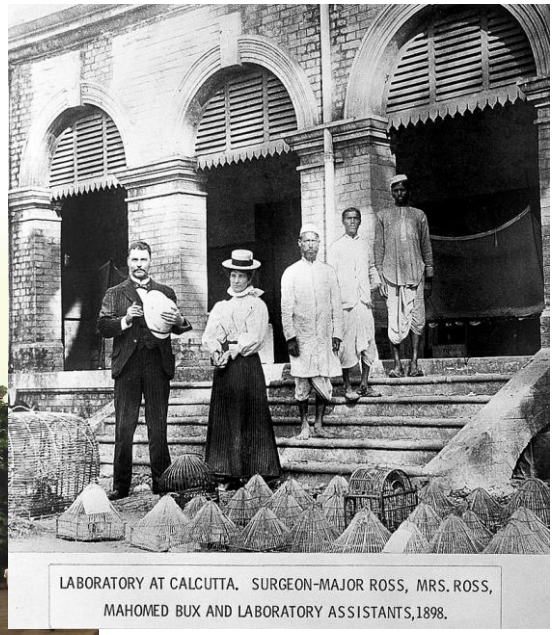
Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization









LABORATORY AT CALCUTTA. SURGEON-MAJOR ROSS, MRS. ROSS, MAHOMED BUX AND LABORATORY ASSISTANTS, 1898.

The disease became known as Leishmaniasis after William Leishman, a doctor serving with the British Army in India , developed one of the earliest stains of Leishmania in 1901. In Dum Dum, a town near Calcutta, Leishman discovered ovoid bodies in the spleen of a British soldier, who was experiencing bouts of fever, anemia, muscular atrophy and swelling of the spleen.

Leishman described this illness as “dum dum fever” and published his findings in 1903. Charles Donovan also recognized these symptoms in other kal-azar patients and published his discovery a few weeks after Leishman. After examining the parasite using Leishman's stain, these amastigotes were known as Leishman-Donovan bodies and officially, this species became known as, L. Donovani. By linking this protozoan with kal-azar, Leishman and Donovan discovered the genus, Leishmanias.

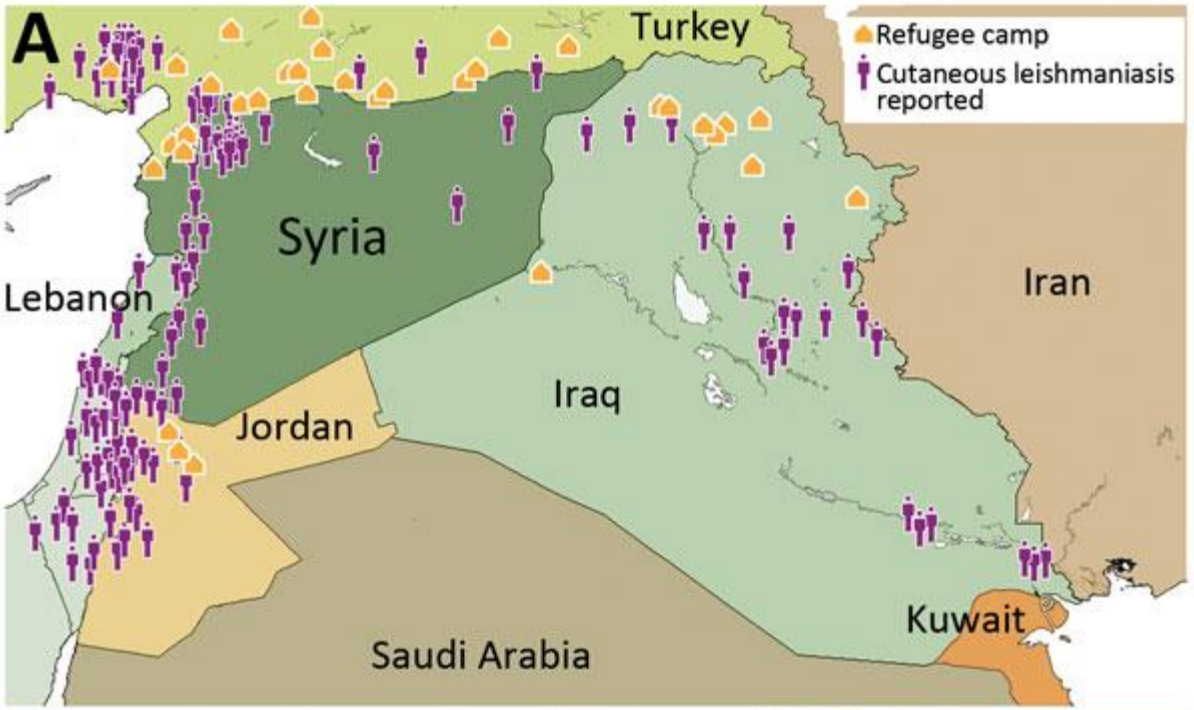
Cutaneous leishmaniasis (CL) is a form of leishmaniasis in which the patient generally presents with one or several ulcer(s) or nodule(s) on the skin, resulting from the infection of phagocytic cells located in the dermis. Most of the twelve million people infected with Leishmania worldwide are CL cases, an 1.5 million new cases occur annually.



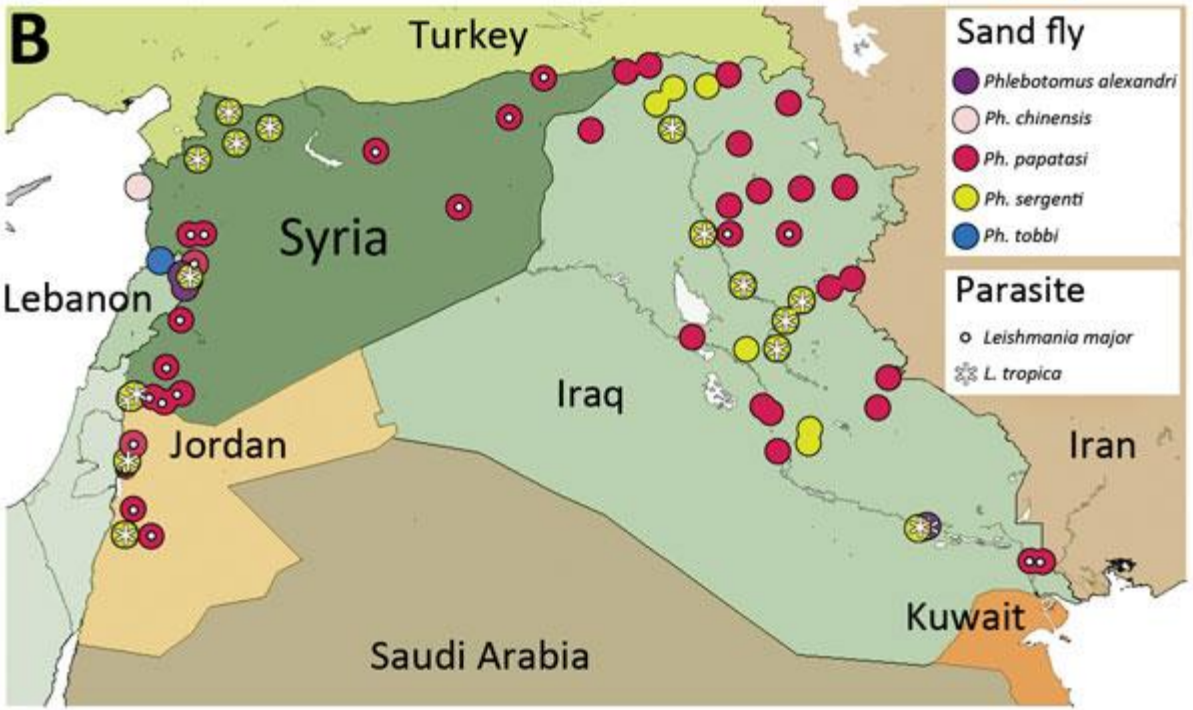


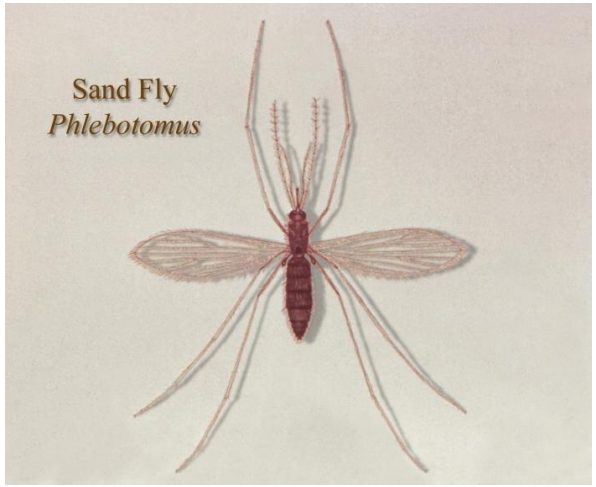


Cutaneous leishmaniasis prevalence within Syria and neighboring countries of the World Health Organization's Eastern Mediterranean Region.



A) Prevalence among refugee camps.





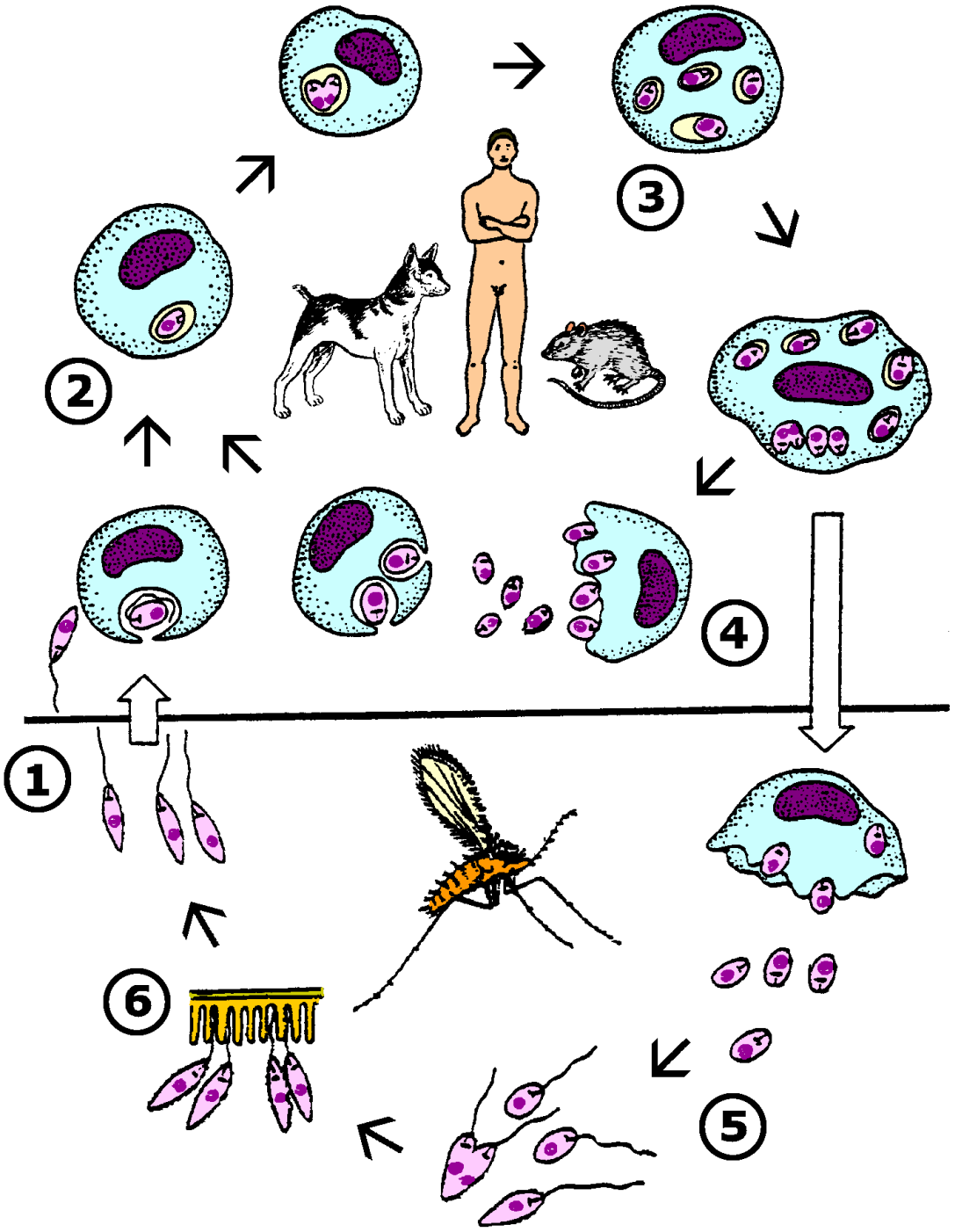
B) Distribution of sand fly and parasite species.

1) metacyclic promastigotes

2) phagocytosis by macrophage → amastigote

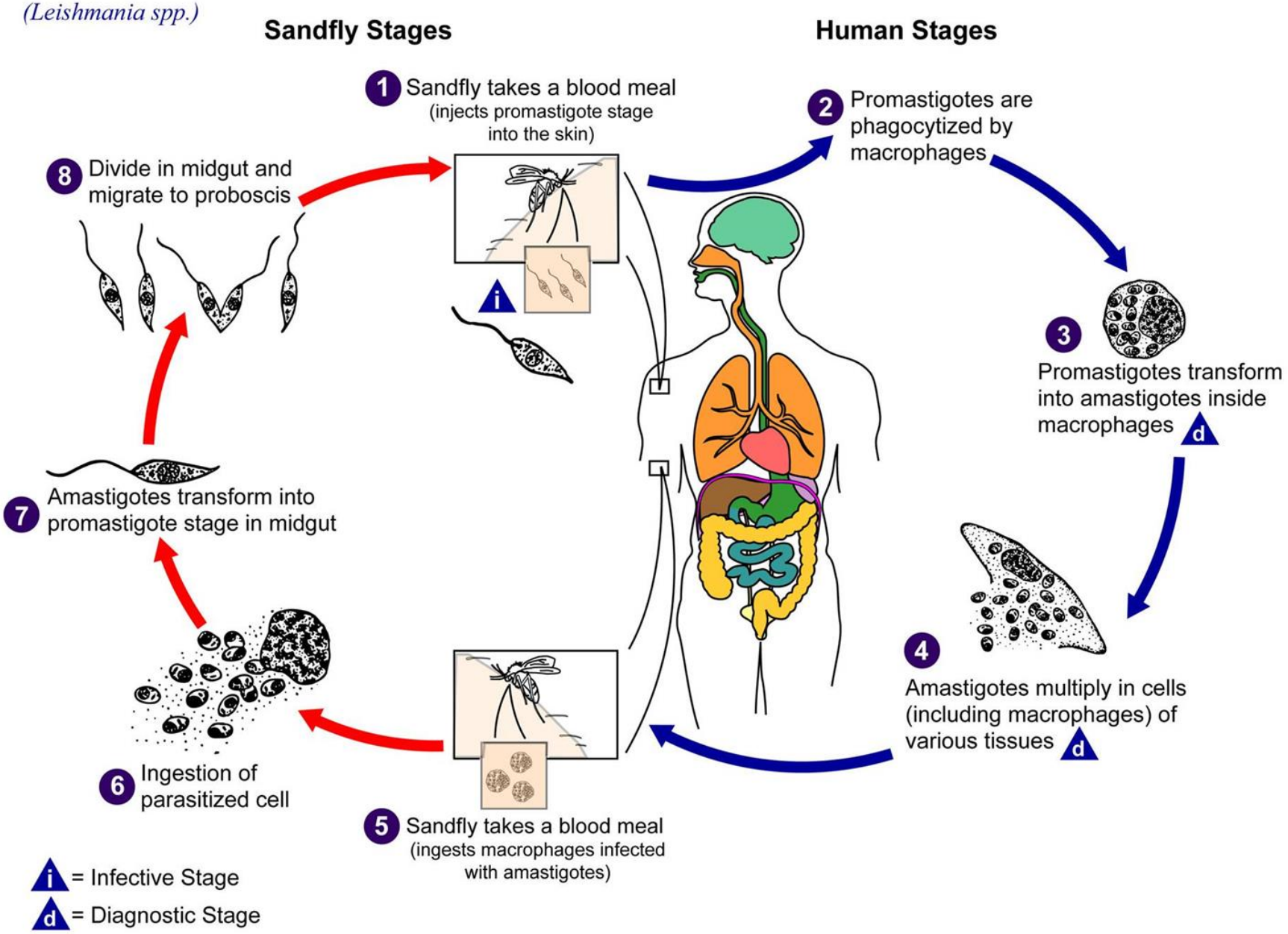
3) replication within macrophage

4) release and phagocytosis of amastigotes



Leishmaniasis

(Leishmania spp.)



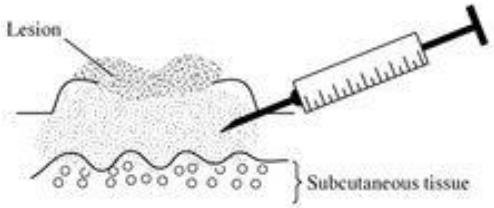
TREATMENT
VISCERAL LEISHMANIASIS

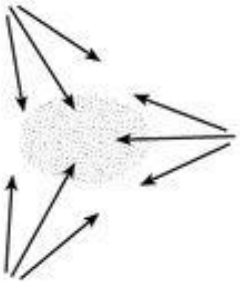
- Liposomal amphotericin-B (AmBisome®) is the drug of choice
  - 3 mg/kg per day on days 1-5, day 14 and day 21
- Pentostam® is an alternative therapy
  - 28 days of therapy is required
- Although AmBisome® is widely available, the difficulty of accurate diagnosis and the potential severity of visceral infection suggest possible patients be referred to the Leishmania Treatment Center at WRAMC for maximal diagnostic efficiency







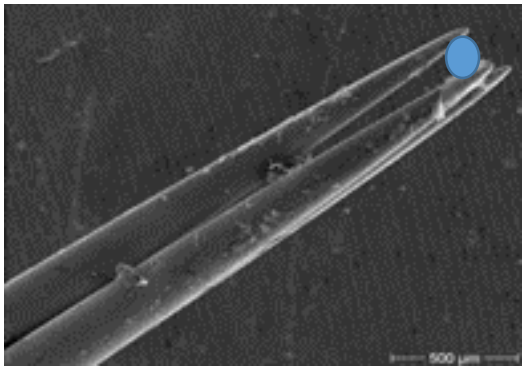




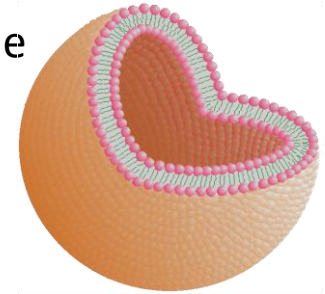
Advance the needle whilst injecting under pressure in the dermis, covering the whole lesion including the centre







A PMU machine typically pushes
particles into the skin towards the
Stratum basale



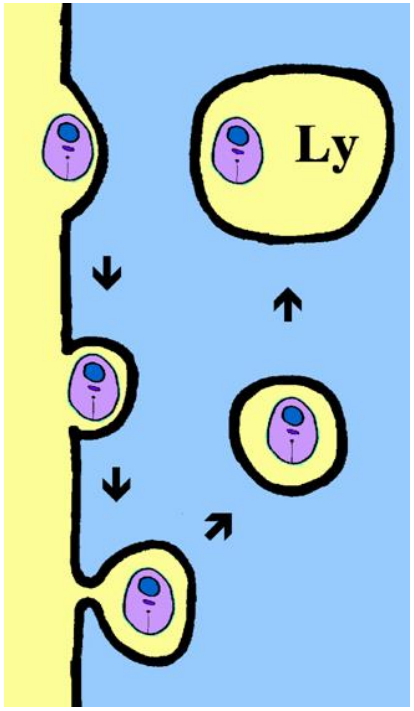
In our study the drug **oleylphosphocholine** (OlPC) was formulated as liposomes, particles known to be prone to macrophage ingestion. Topical delivery of this new drug formulation was used to maximize efficacy.
For efficient topical drug delivery in the skin a Permanent Make Up (PMU; 'AmieaMed') machine was used, which causes less pain compared to the classical injection with syringes of therapeutic substances.

Leishmania-Macrophage Interactions

When an infected Phlebotomid sand fly takes a blood meal it infects the vertebrate host with promastigote metacyclic forms. Within a short time the promastigotes are taken up by macrophages, the first line of defense of the immune system. During the process of uptake by the macrophage, the promastigote loses its flagella and transforms into the amastigote form. Once internalized in a phagosome the macrophage lysosome fuses with the phagosome to from a phagolysosome containing the parasite.

How does the parasite survive this hostile environment?

- **attachment and entry**
  - **involves CR3 and surface molecules on parasite (Lipophosphoglycan)**
  - **entry is typical phagocytosis**
  - **phagosome fuses with lysosome**

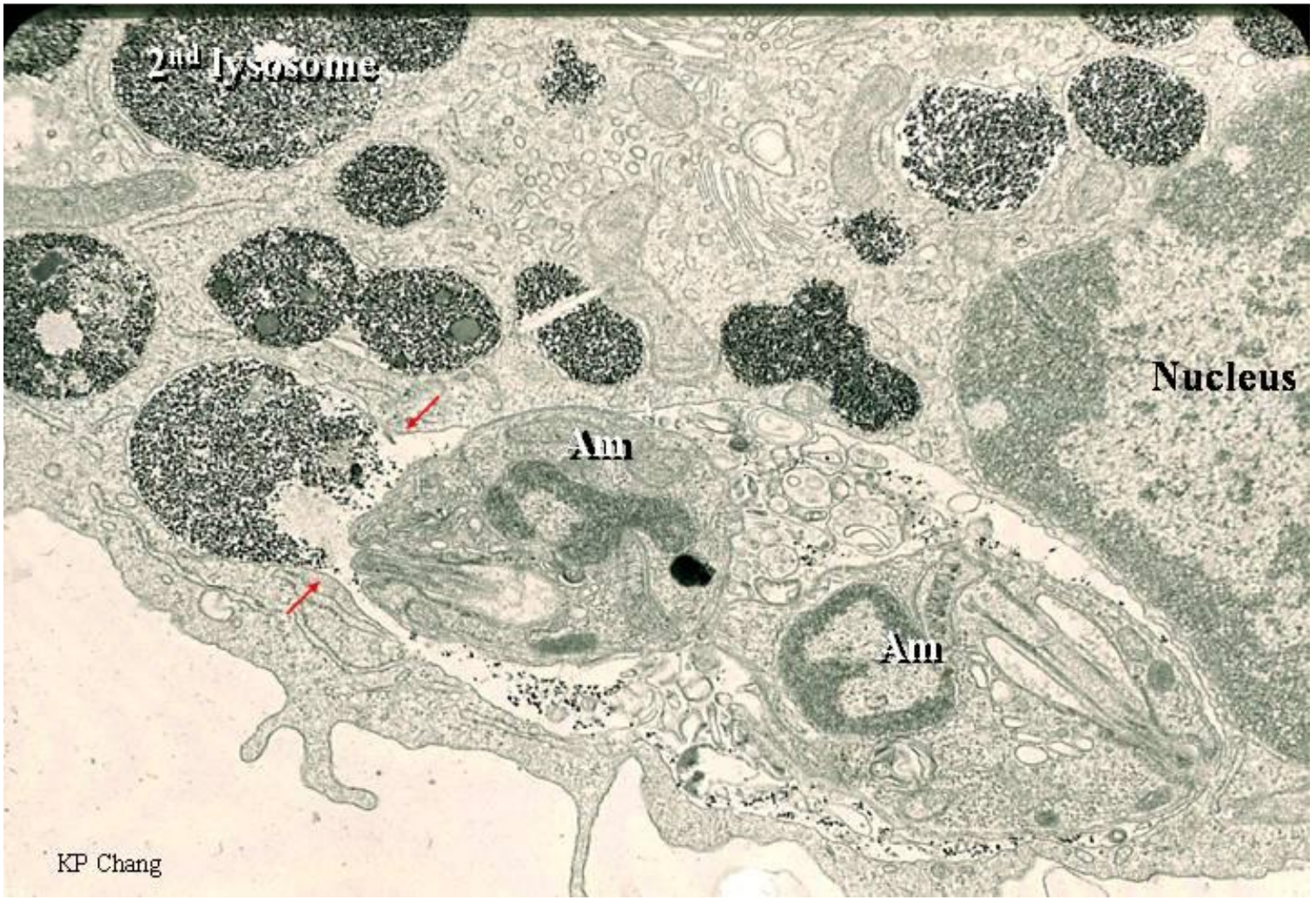


SURVIVAL IN THE MACROPHAGE PHAGOLYSOSOME

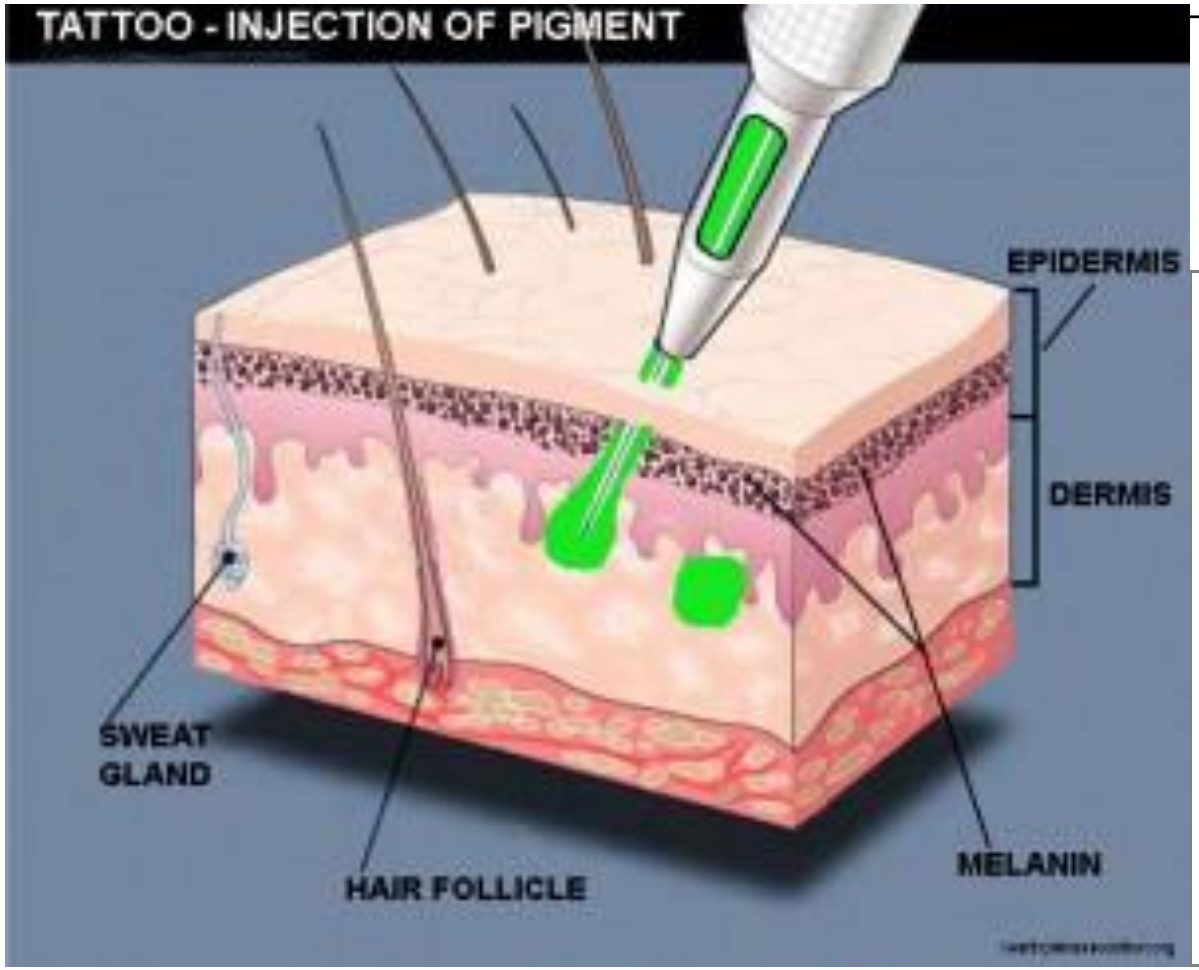
Respiratory burst products:

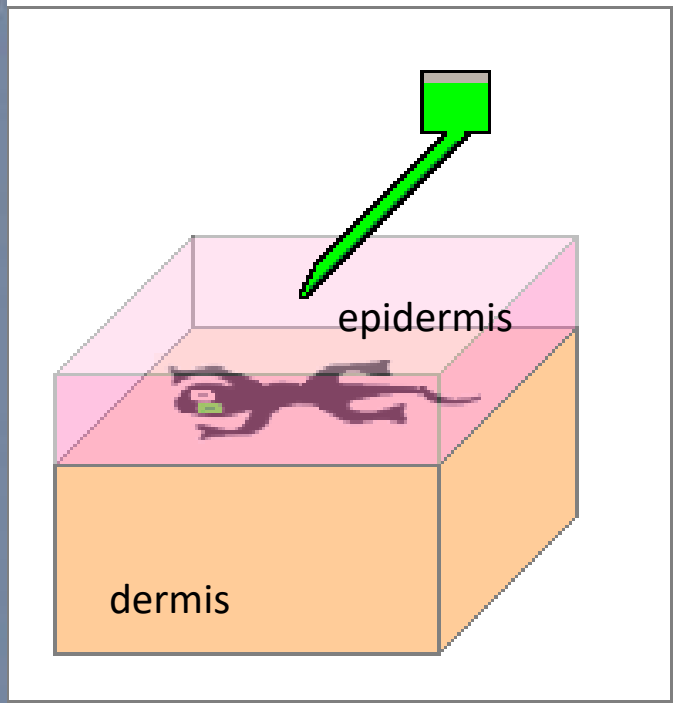
H2O2, superoxide hydroxyl radicals

Phagosome-lysosome fusion in *L donovani* infection of macrophage

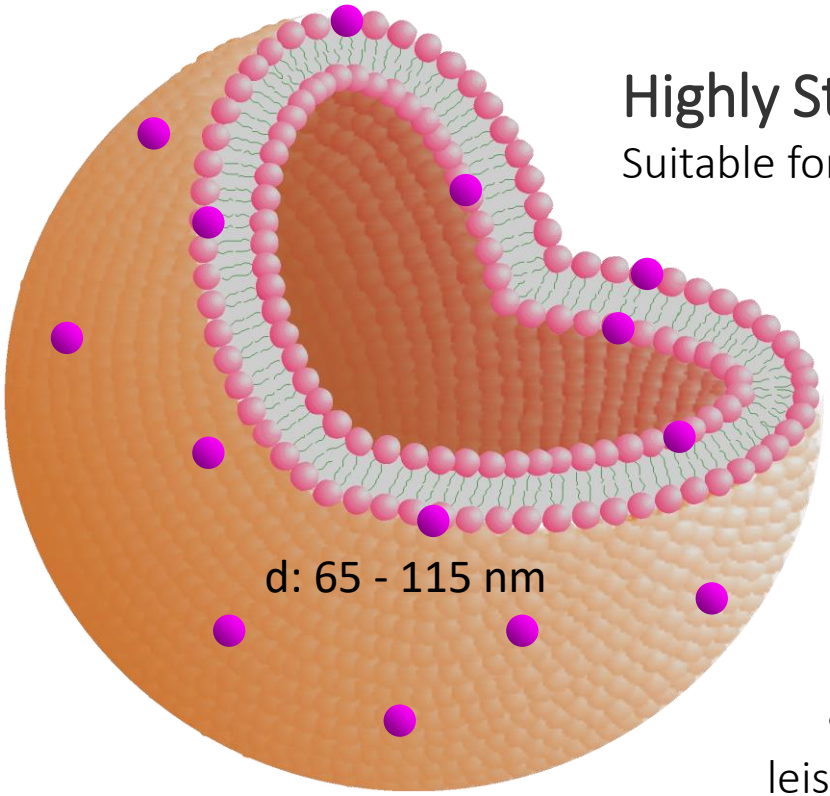


Bringing pigments into the skin





Medical application: Cutaneous leishmaniasis treatment



Highly Stable

Suitable for distribution in developing countries

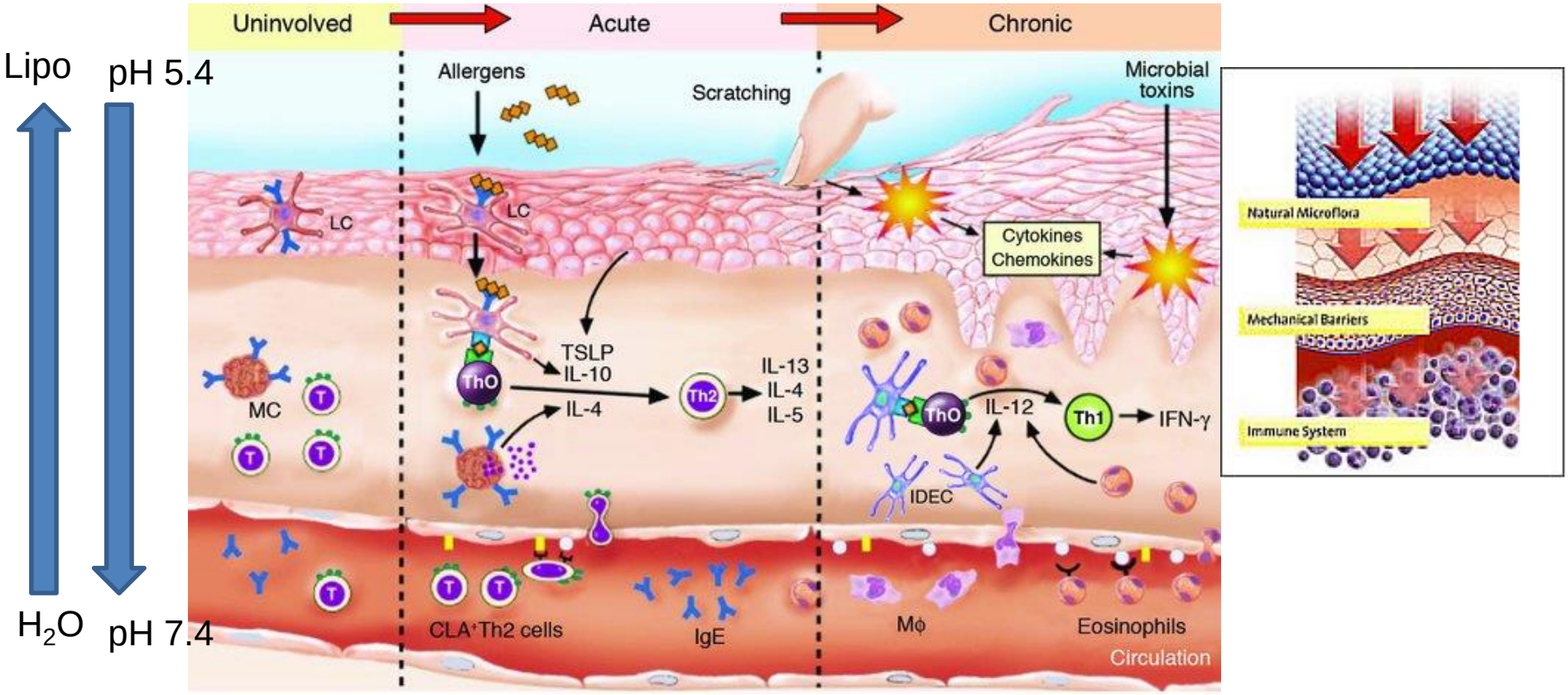
Low cost process

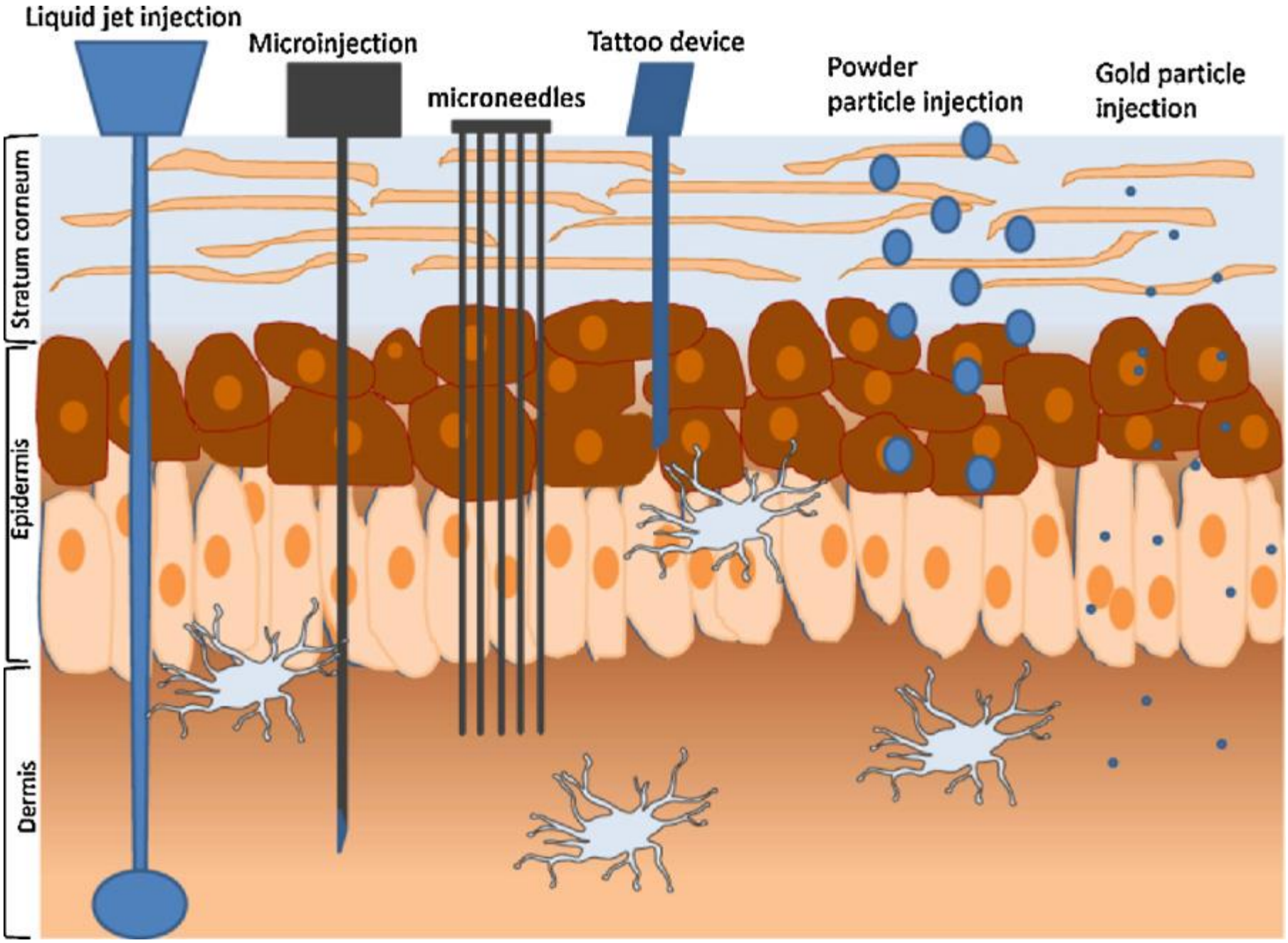
Will translate into low cost treatment

Multiple possible applications

- Intravenous injection to treat visceral leishmaniasis
- Transdermal formulation to treat cutaneous leishmaniasis
- Paediatric liquid formulation
- Administration via PMU device

Skin is our most important barrier against the outer (microbial& chemical) world



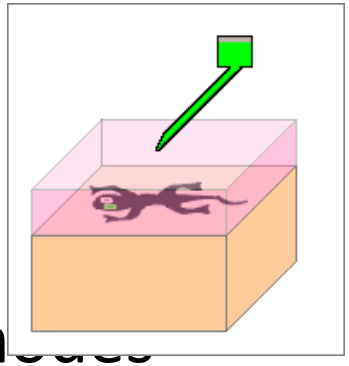

Liquid jet injection
Microinjection
microneedles
Tattoo device
Powder particle injection
Gold particle injection
Stratum corneum
Epidermis
Dermis



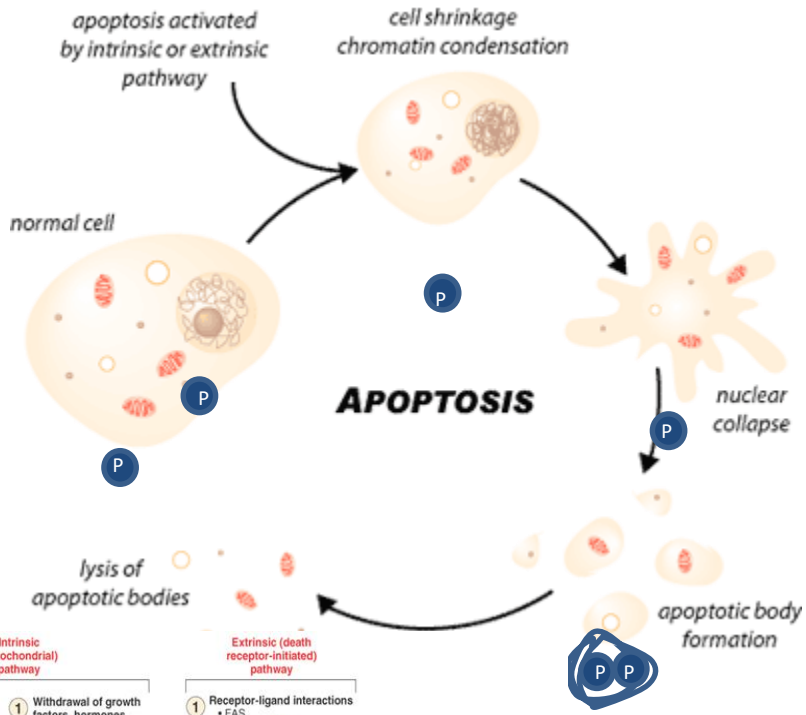


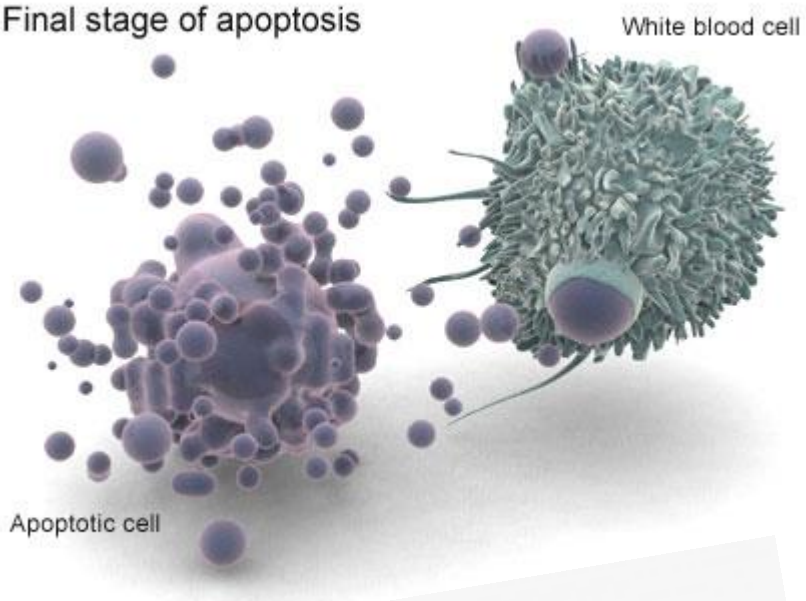
Where goes the particle?

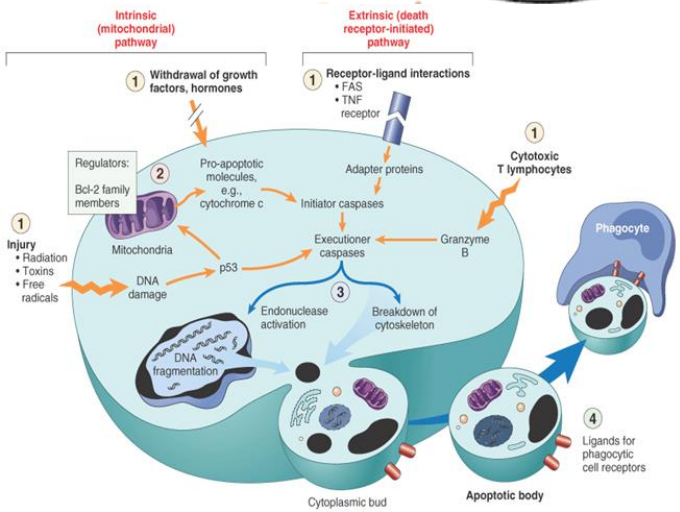
- Strange body
- Neutrophil activation (innate immune system)
- Macrophages
- Fagocytose
- Apoptose
- To move or not to move => Lymph nodes
- Encapsulation
- Life-long present

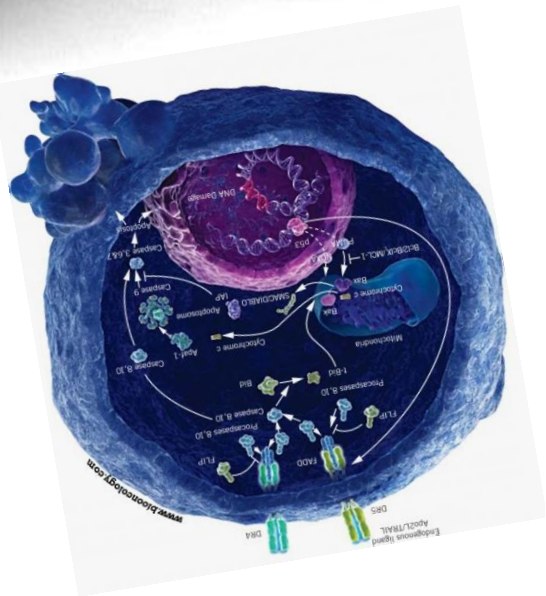


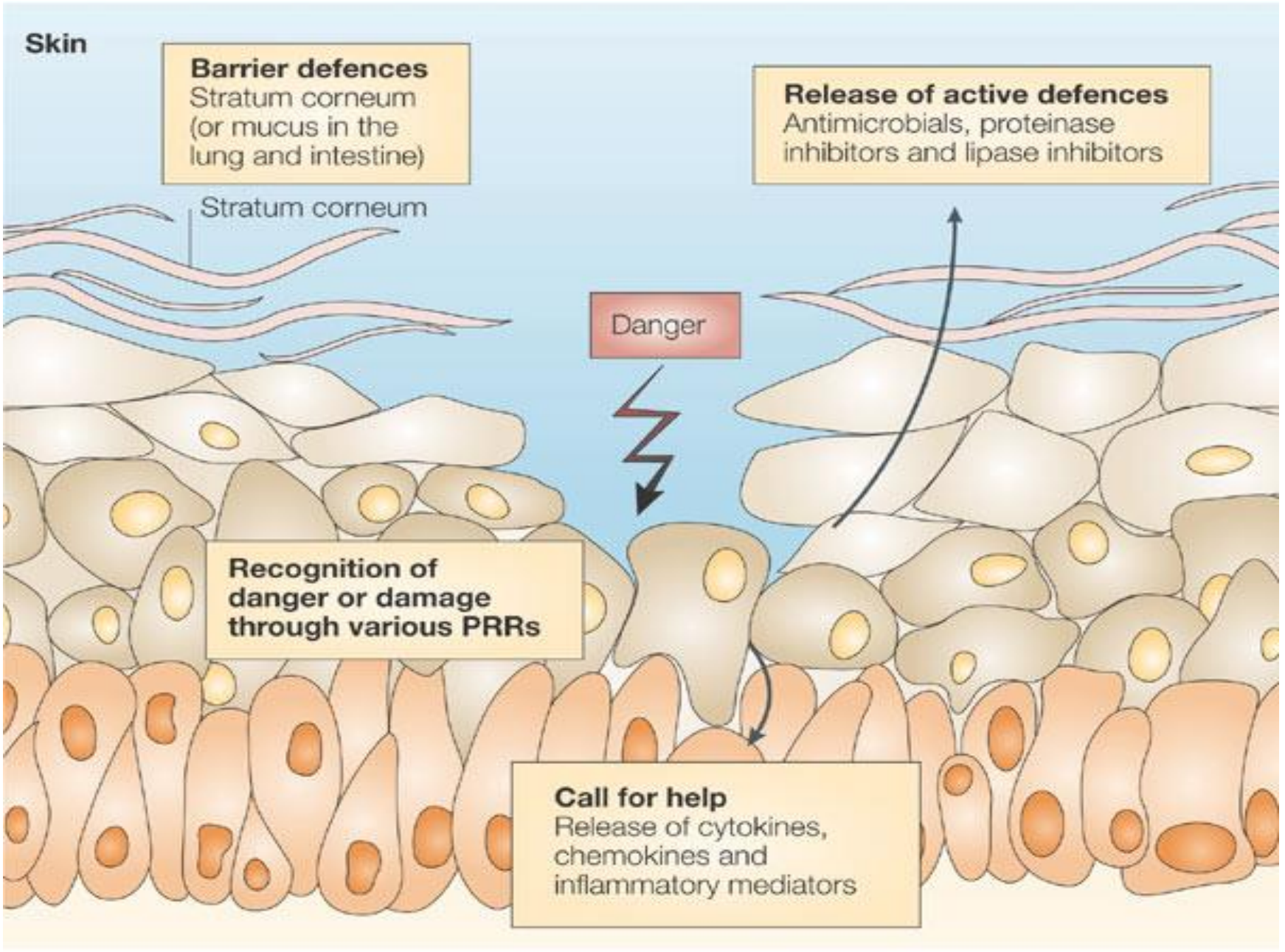
Apoptosis


apoptosis activated by intrinsic or extrinsic pathway
cell shrinkage chromatin condensation
normal cell
APOPTOSIS
nuclear collapse
lysis of apoptotic bodies
apoptotic body formation
P


Final stage of apoptosis
White blood cell
Apoptotic cell
U.S. National Library of Medicine


Intrinsic (mitochondrial) pathway
Extrinsic (death receptor-initiated) pathway
1 Withdrawal of growth factors, hormones
1 Receptor-ligand interactions
• FAS
• TNF receptor
Regulators:
Bcl-2 family members
2
Pro-apoptotic molecules, e.g., cytochrome c
Adapter proteins
Initiator caspases
1 Cytotoxic T lymphocytes
Mitochondria
Executioner caspases
Granzyme B
Phagocyte
1 Injury
• Radiation
• Toxins
• Free radicals
DNA damage
p53
3
Endonuclease activation
Breakdown of cytoskeleton
DNA fragmentation
4 Ligands for phagocytic cell receptors
Cytoplasmic bud
Apoptotic body

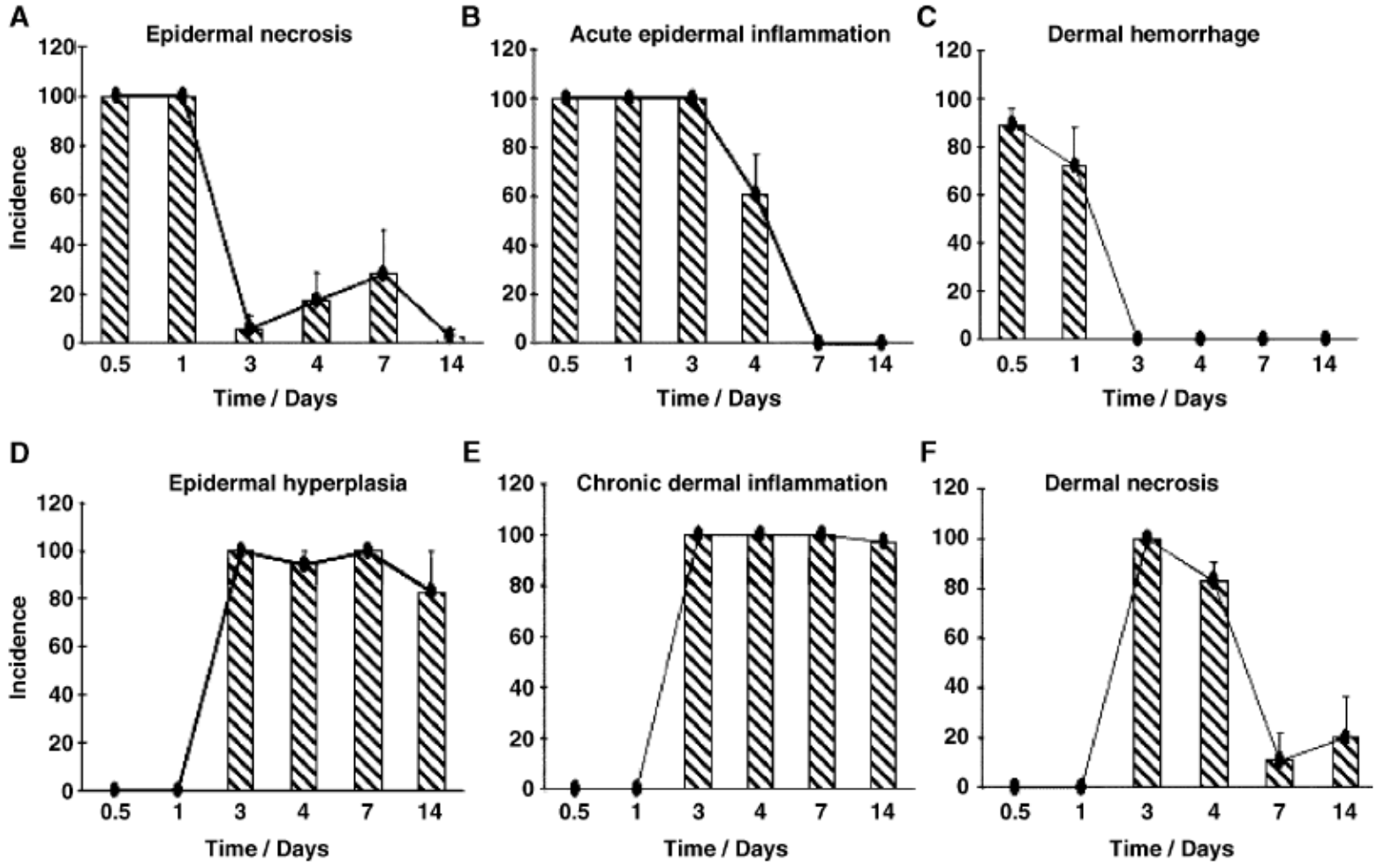




Nature Reviews | Immunology

Reactions seen after tattooing

N.V. Gopee et al. / Toxicology and Applied Pharmacology 209 (2005) 145–158



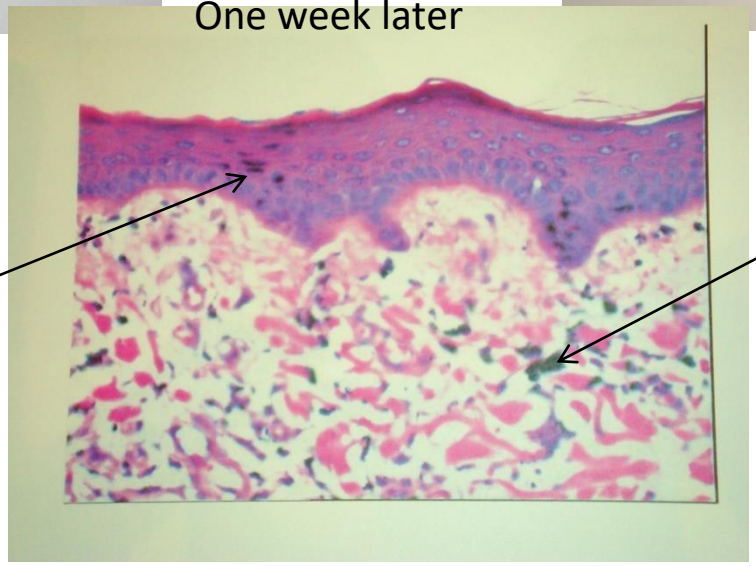
Prof. Jens Teifke, Insel Riems



Fresh tattoo



One week later

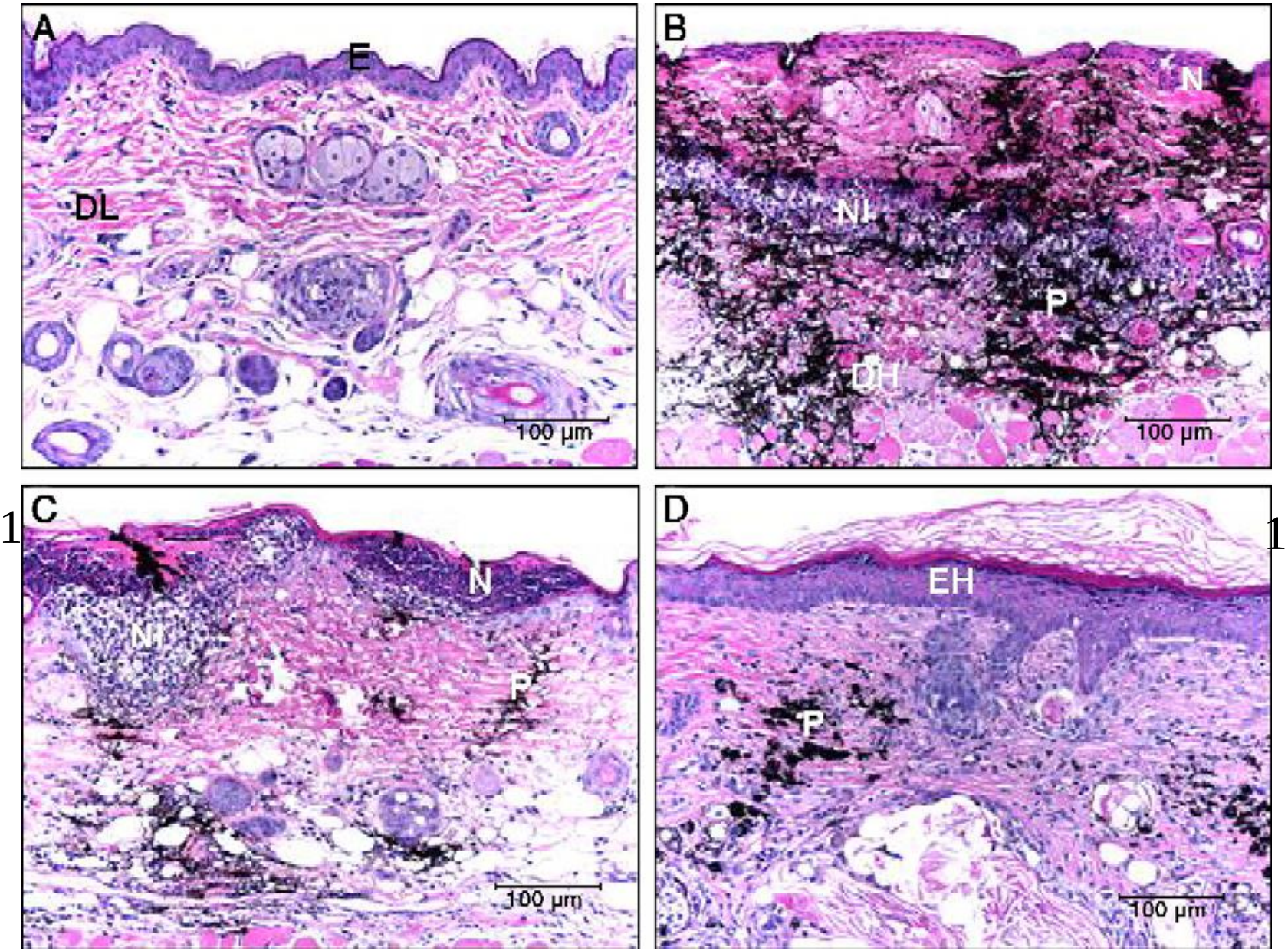


Coloured macrophages
(do not disappear)

Coloured keratonicytes
(will disappear in time)

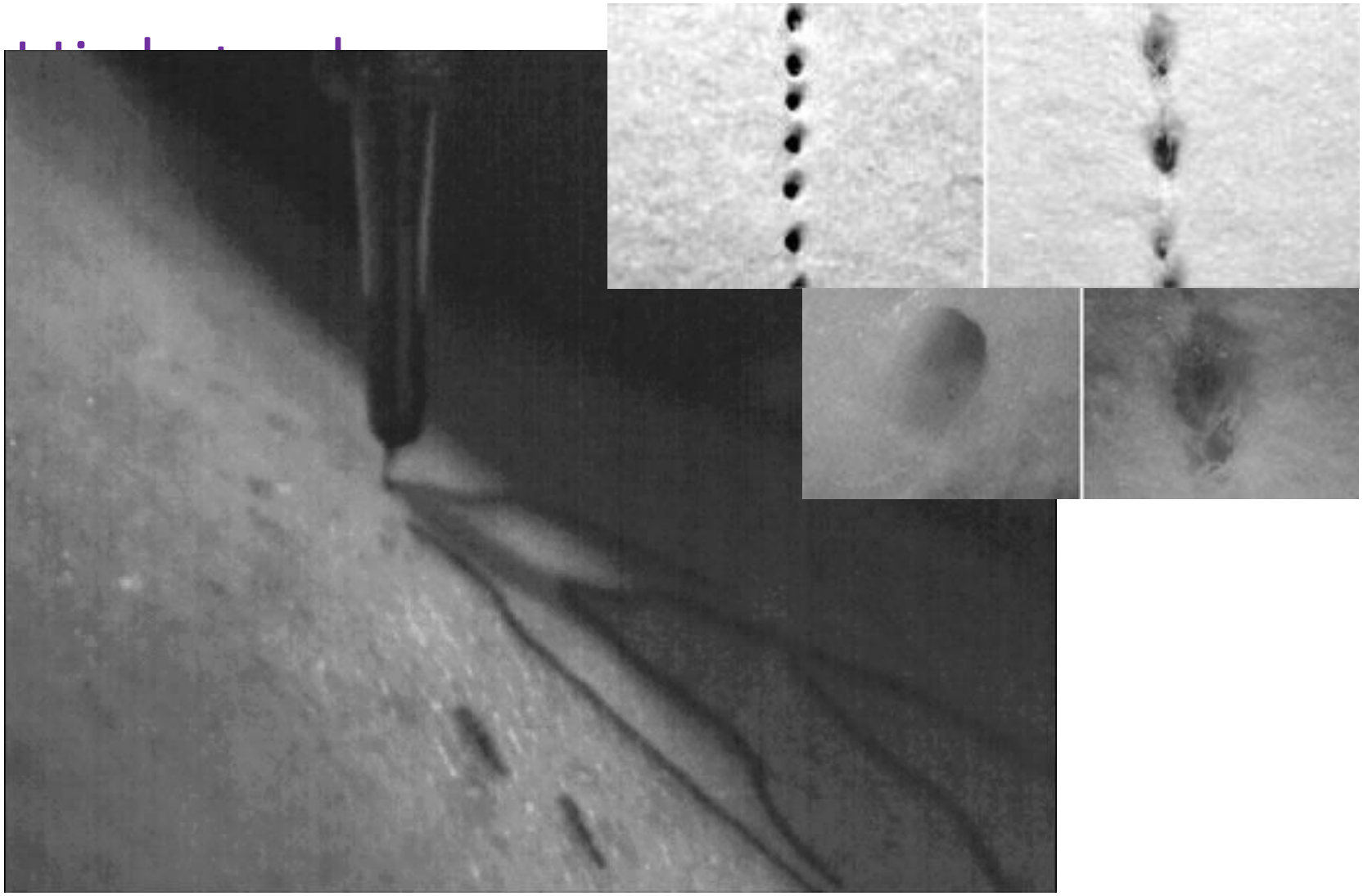
Untreated

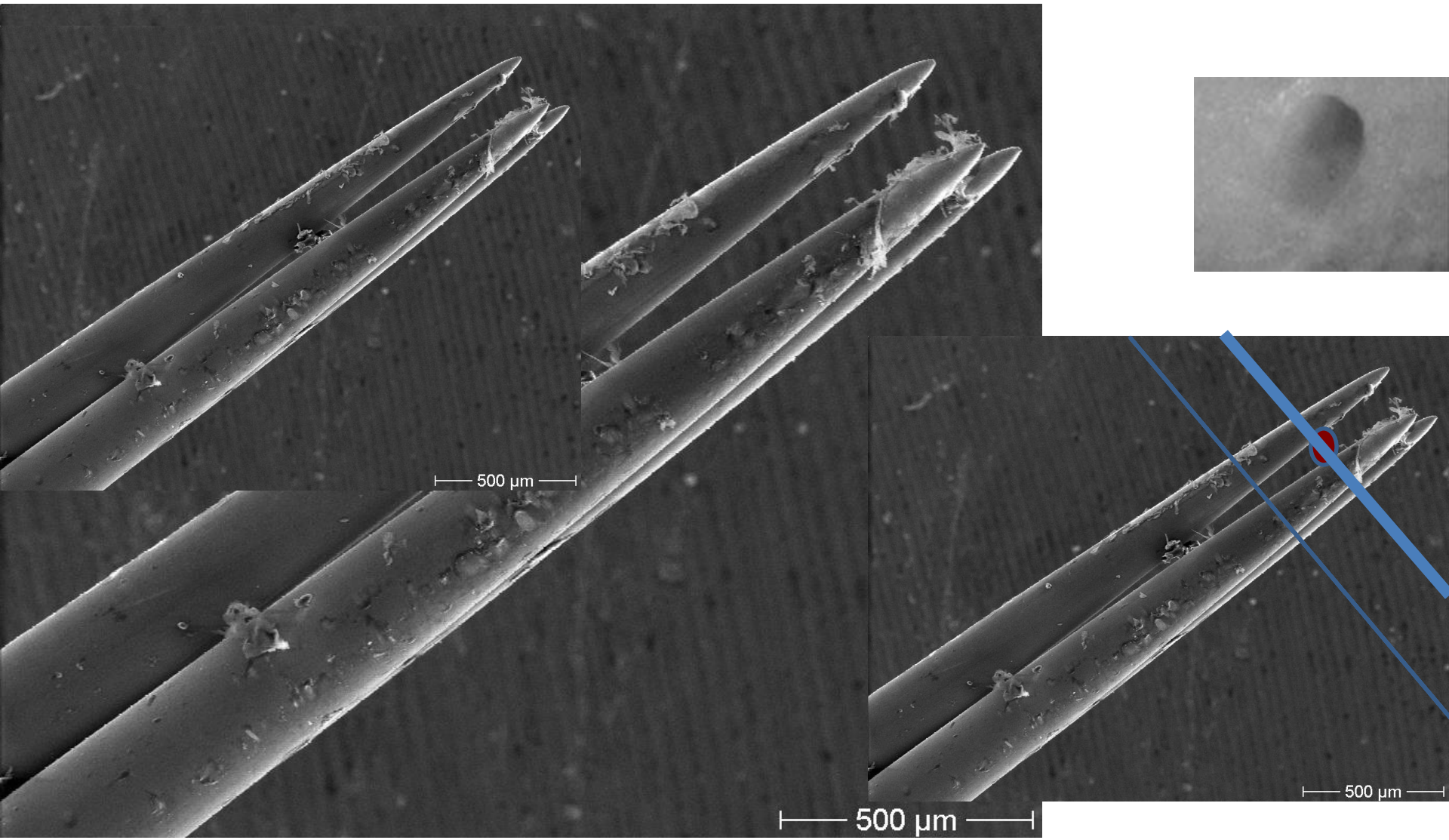
After half a day



Day 1

14 d




500 µm
500 µm
500 µm

Safety testing on pigs



2) Tattooing drug particles on healthy pig skin does not cause any adverse reaction

The pig is a recognized and accepted toxicological model for intradermal studies as the skin physiology in mini pigs is more similar to that of humans compared to other animal species.

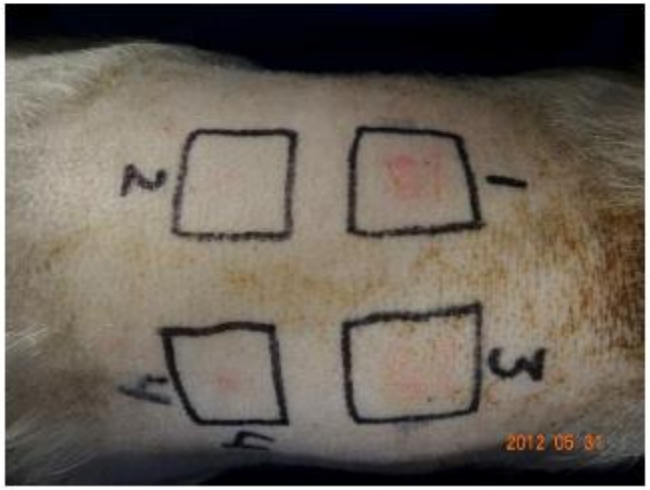
A



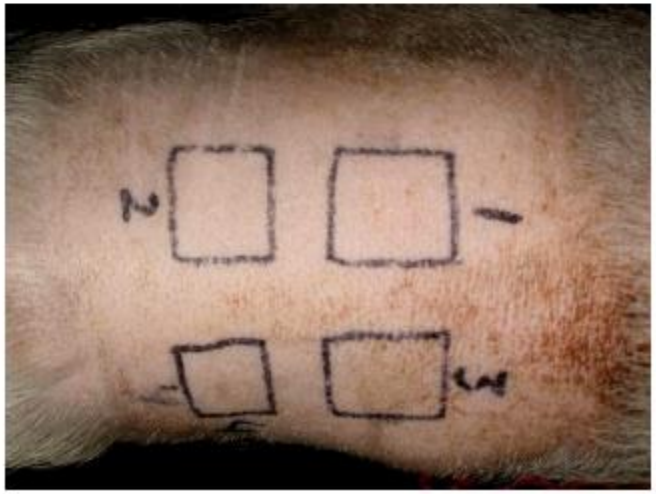
B



C



D



3) Tattooing makes particles reach dermal macrophages, which are the cells infected by *Leishmania* parasites (Figure 5B)

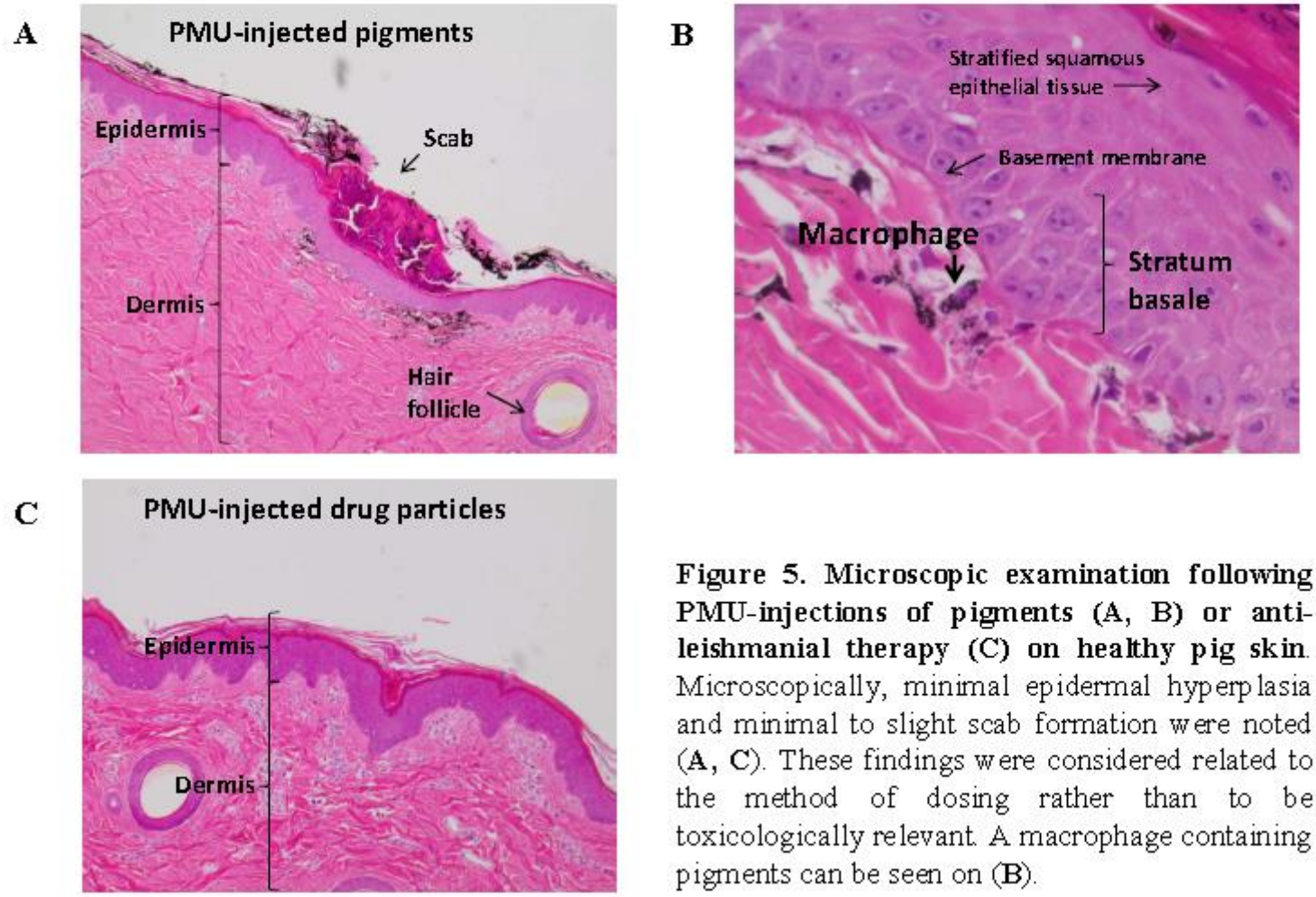
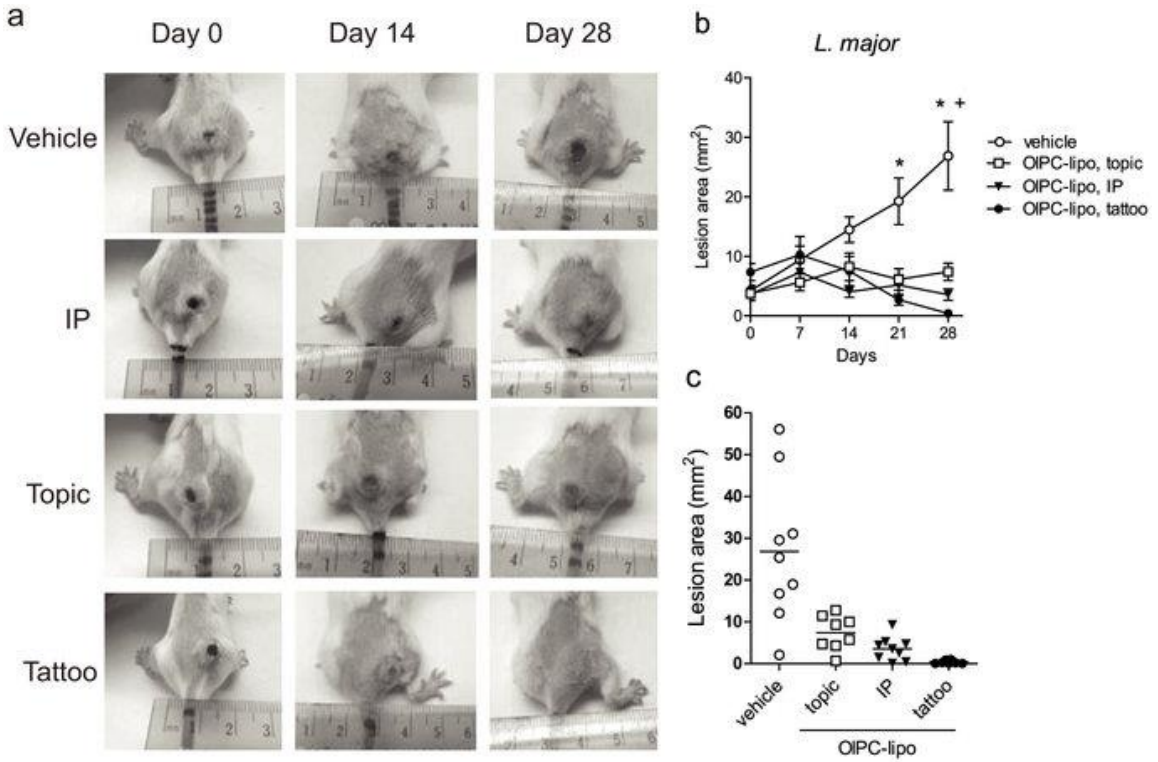


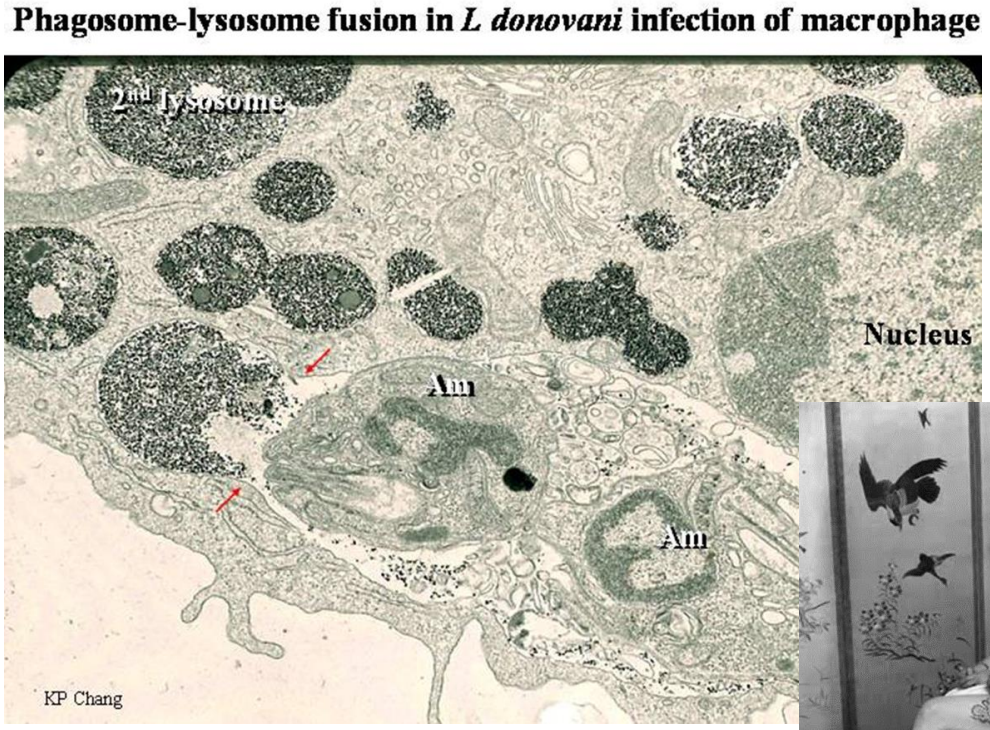
Figure 5. Microscopic examination following PMU-injections of pigments (A, B) or anti-leishmanial therapy (C) on healthy pig skin. Microscopically, minimal epidermal hyperplasia and minimal to slight scab formation were noted (**A, C**). These findings were considered related to the method of dosing rather than to be toxicologically relevant. A macrophage containing pigments can be seen on (**B**).



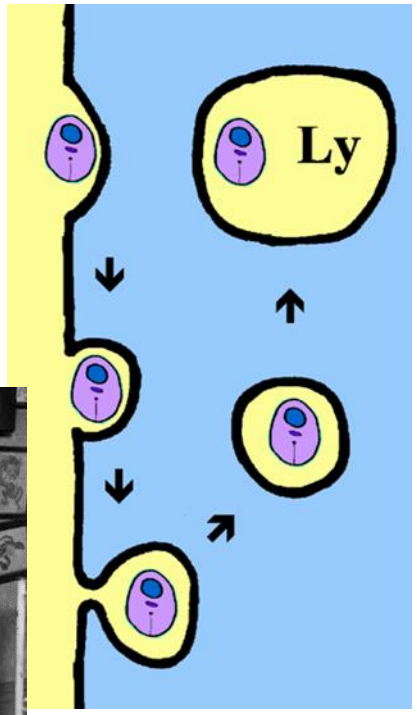
This first example of tattoo-mediated drug delivery could open to new therapeutic interventions in the treatment of skin diseases.

First was shown that treatment of cultured Leishmania-infected macrophages with OlPC-liposomes results in a direct dose-dependent killing of intracellular parasites. Based on this, in vivo efficacy was demonstrated using a 10 day tattooing-mediated treatment in mice infected with L. major (left) and L. mexicana (not shown). In both models this regimen results in rapid clinical recovery with complete regression of skin lesions by Day 28. Parasite counts and histopathology examination confirm high treatment efficacy at the parasitic level. Low amount of drug required for tattooing combined with fast clinical recovery has positive impact on CL patient management.

This study demonstrates that the use of a tattoo instrument for drug delivery is possible in the treatment of cutaneous leishmaniasis, and that this method can successfully eliminate intracellular parasites at the site of infection.







Three routes of administrations of the same drug formulation were investigated:

- Systemic (IP) administration
- Topical administration as a drop
- Administration via a tattoo instrument

Evaluation parameters included clinical (lesion sizes) and parasitological parameters (burdens) using quantitative and qualitative methods.
In all experiments, the tattooing delivery procedure was the most efficacious at both the clinical and parasitological levels.





Limitations

The used tattoo device, used routinely for permanent make-up procedures, is not yet optimal for quantitative drug delivery.
New techniques are under development to make the procedure with a PMU machine a more quantitative controllable procedure.

Any questions?



Civil-Military-Interaction Command

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